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Letter to the Editor

Galectin-6 is a novel skin anti-microbial peptide that is modulated by the skin barrier and microbiome☆

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To the Editor,

We have previously reported that mice lacking three components of the epidermal cornified envelope – Envoplakin, Periplakin and Involucrin – (EPI−/− mice) exhibit several hallmarks of atopic dermatitis and are resistant to developing chemically induced skin tumours [1,2]. EPI−/− mice have an enhanced skin bacterial load and exhibit upregulation of a variety of anti-microbial proteins (AMPs), including β-defensins and S100A9 [3].

When analysing a previously published dataset of genes that are differently expressed in the skin of EPI−/− and wild type (WT) mice, we noticed a more than 3-fold increase in expression of Galectin-6 [2]. Galectins are a family of lectins with binding specificity for β- galactosides [4]. Although the function of Galectin-6 has not been characterized, several galectin family members have antimicrobial activity, targeting blood group positive microbes [5]. This led us to hypothesise that Galectin-6 is a novel skin AMP.

When EPI−/− mice are housed under conventional conditions, the skin microbiota, as quantitated by 16 S ribosomal RNA levels, is approximately three-fold more abundant than in WT controls [3] (see Supplementary Information for details of mice and experimental techniques). However, when EPI−/− mice are housed under specific pathogen-free (SPF) conditions the bacterial load falls to that of WT mice [3]. We confirmed that Galectin-6 mRNA was significantly higher in EPI−/− mice (Fig. 1A). Furthermore, in contrast to the AMPs analysed previously [3], Galectin-6 expression was decreased in the skin of EPI−/− mice housed under SPF conditions (Fig. 1A).

By comparing levels of Galectin-6 in total skin, from which the adipocyte layer had been removed, and epidermis, we could show that Galectin-6 is more abundant in the dermis than the epidermis (Fig. 1A). This is of interest, given that bacteria can penetrate the living epidermal layers in EPI−/− mice [3]. Since galectin-6 gene expression in WT skin was not altered when WT mice were housed under SPF conditions (**Supplementary** Fig. 1), galetin-6 is likely to respond to an abundant skin flora when the epidermal barrier is defective, but not to microbial load under steady state conditions.

Amongst galectin family members, galectin-4 shares more than 80% sequence homology with galectin-6. Lgals6 represents a duplication of the galectin-4 gene, and the two genes share 8 out of 10 exons [6]. However, expression of other galectins, including galectin-4, did not differ between EPI−/− and WT whole skin, irrespective of whether or not the EPI−/− mice were maintained under SPF conditions (Fig. 1B).

Given its unusual expression in EPI−/− skin, we investigated whether Galectin-6 has anti-microbial activity. A range of bacterial strains were grown in the presence of recombinant Galectin-6 or control protein expressing GST tag only. As previously reported for Galectin-4 and Galectin-8 [7], Galectin-6 suppressed the growth of E. coli expressing blood group antigen (*E. coli* O86, possessing blood group-B antigen), in a dose-dependent manner (Fig. 1C), and did not affect the growth of non-blood group antigen-expressing E. coli or other bacterial strains tested (Fig. 1D). The antimicrobial activity of galectin-6 was similar to that of murine galectin-4 (Supplementary Fig. 2). We conclude that Galectin-6 is a novel AMP, the expression of which can be modulated by skin bacterial load.

Galectin-6 differs from the other AMPs we have examined in being regulated by bacterial load in EPI−/− skin. This is all the more remarkable, given that its abundance is low in the epidermis relative to total skin. Lgals6 is also unusual in that it does not have a human homologue and is present in some mouse species, including 129sv, but not in others, such as C57BL/6 and Balb/c [6]. Even though Galectin-4 and Galectin-6 are highly conserved, their expression patterns in the digestive tract differ following challenge with dextran sodium sulfate [8]. This is consistent with the differential regulation of Lgals6 and Lgals4 by the skin microbiota. Furthermore, previously published microarray data show that Lgals4 expression is not significantly altered in atopic dermatitis and psoriatic skin compared with normal controls (GSE26952) [9]. We cannot exclude the possibility that skin barrier defects also contributed to the upregulated expression of Lgals6 in EPI−/− mice, because the level in flora-deficient EPI−/−mice was still higher than that of controls. Nevertheless, our findings reveal that Galectin-6, unlike other AMPs [3], is influenced by the abundance of skin microbiota. We also do not rule out additional functions for Galectin-6 in the skin; for example in the dermis it could potentially modulate fibrosis, as in the case of galectin-3 in the lung [10].

Abbreviations: EPI−/−, lacking Envoplakin, Periplakin and Involucrin; AMPs, anti-microbial proteins; WT, wild type; SPF, specific pathogen-free; GST, Glutathione S-transferase.

☆ The work was performed at Hokkaido University, Japan, and Cambridge University, UK.

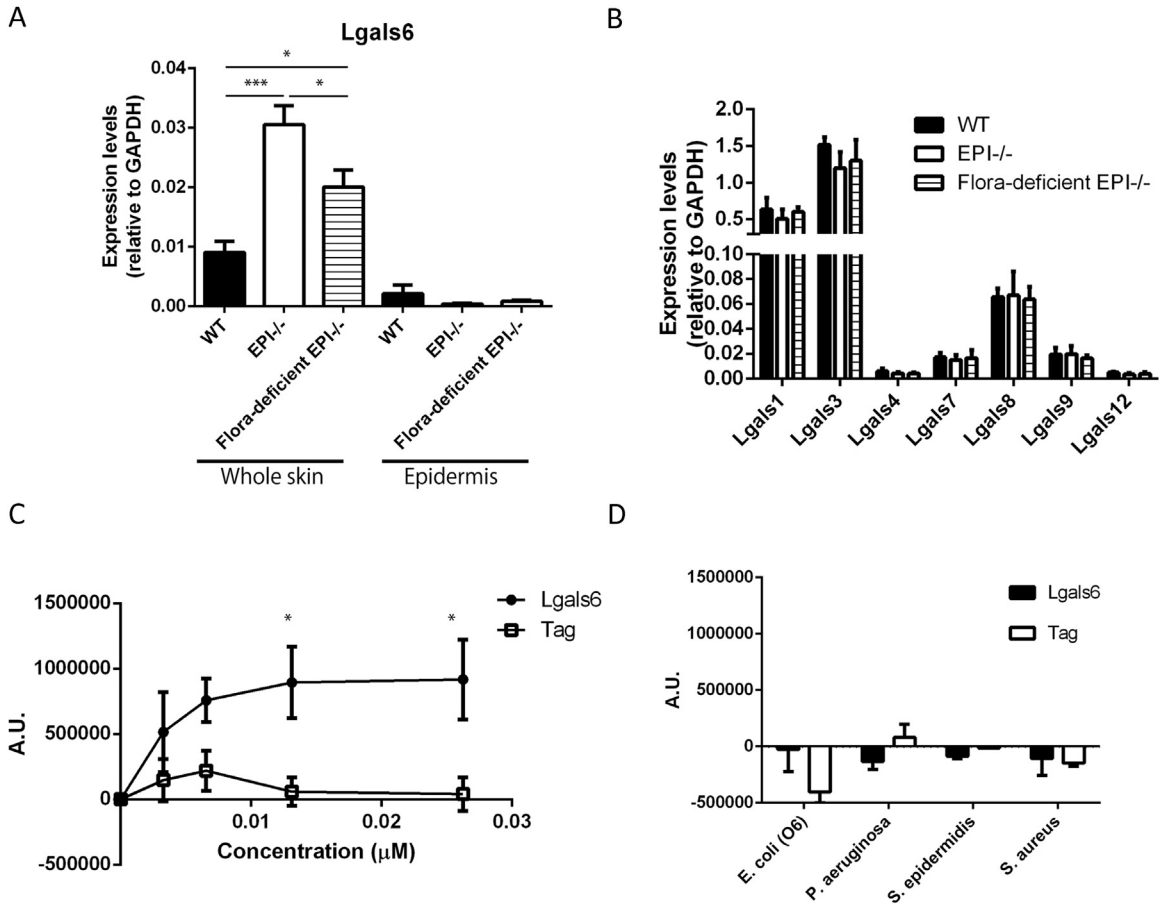

Fig. 1. Lgals6 expression and function in skin (A) qRT-PCR of Lgals6 in WT, EPI−/− and flora-deficient EPI−/− skin and epidermis. (B) qRT-PCR of genes encoding other galectins (Lgals1, Lgals3, Lgals4, Lgals7, Lgals8, Lgals9 and Lgals12) in WT, EPI−/− and flora-deficient EPI−/− whole skin. Data are means ± SEM from at least 4 mice per group. (C) Antimicrobial properties of recombinant galectin-6 (closed circles) or tag protein (open squares) on blood group antigen-expressing E.coli (O86) growth. (D) Growth of non-blood group antigen-expressing E.coli (O6), P. aeruginosa, S. epidermidis and S. aureus treated with the recombinant galectin-6 or tag protein. See supplemental information for methodology and reagents. P-values are indicated with: * $p < 0.05$, *** $p < 0.001$.

Conflict of interest

The authors state no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jdermsci.2016.06.008.

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Letter to the Editor

Changes in tight junction protein expression in intrinsic aging and photoaging in human skin *in vivo*

Tight junctions (TJs) exist in a planar network and function as barriers that seal the intercellular space between epithelial cells to prevent water loss [1]. TJs contain protein complexes, are situated in the epidermal granular layer, and are composed primarily of claudins, occludin, and ZO proteins, which act in a systematic manner and regulate TJ function [1]. In addition to their role as water barriers, bidirectional paracellular penetration of ions and proteins is possible through TJs. Moreover, in response to various environmental stresses, TJs are dynamically regulated and interact with Langerhans cells [2]. Skin aging is a complex process induced by intrinsic and/or extrinsic factors, like ultraviolet (UV) radiation, and is accompanied by physiological changes in the skin that involve impaired barrier function [1]. Since changes in major TJ components in aged skin have not been characterized, we aimed to elucidate changes in TJ protein expression in intrinsic aging and photoaging.

To examine effects of aging on TJs, expression of three major TJ components (claudin-1, occludin, and ZO-1) was evaluated in intrinsically aged and photoaged human skin. Photoprotected buttock and photoaged forearm skin tissues were collected from elderly volunteers (n = 5, median age: 77 years, interquartile range: 75–81) and young volunteers (n = 5, median age: 28 years, interquartile range: 26–31). Because TJs also exist in endothelial cells in the dermis, the epidermis was separated from the dermis by incubating whole skin tissue at 55 °C in phosphate-buffered saline (PBS) for 2 min and physically peeling off the epidermis. Western blotting and quantitative polymerase chain reaction (PCR) were performed using the epidermis, and immunofluorescence (IF) staining was conducted using the whole skin. IF data were graded according to the intensity within the granular layer by three expert dermatologists who were blinded to the identity of each image. This study was approved by the Institutional Review Board at Seoul National University Hospital, and was conducted according to the Declaration of Helsinki; written informed consent was received from all subjects.

In intrinsically aged skin, claudin-1 and occludin were significantly downregulated, as shown by western blotting and immunostaining. However, their mRNA levels were not significantly altered (Fig. 1A and B). The staining intensity and area of claudin-1 expression were markedly reduced in samples from elderly participants than in those from young participants. Specifically, within the granular layer, claudin-1 was also significantly decreased in samples from elderly subjects (Fig. 1C). However, there were no significant differences in ZO-1 protein expression between young and aged samples.

To investigate the influence of photoaging on TJs, we compared TJ protein expression in buttock skin with that in matched forearm skin from the same elderly subjects. Claudin-1 expression was significantly decreased at both protein and mRNA levels (Fig. 2A and B). Immunostaining also revealed that claudin-1 was reduced more in photoaged skin than in intrinsically aged skin; thus, claudin-1 was detected sparsely within the upper suprabasal layers in photoaged skin of elderly individuals (Figs. 1C and 2C). In contrast, no significant changes were found between buttock and forearm skin from young individuals (Fig. S1). Photoaged skin exhibited greater decreases in claudin-1 expression compared to intrinsically aged skin (84.9% versus 56.2% reduction, respectively). The other two proteins, occludin and ZO-1, did not show any significant changes in photoaged skin.

We found that claudin-1 was reduced in both intrinsically aged and photoaged human skin *in vivo*. Despite the uncertain role of occludin in barrier function, claudin-1 is known to be important in the formation of functional TJs and recruitment of occludin to TJs, suggesting that impaired claudin-1 expression in aged skin causes the loss of TJ functionality [3]. Moreover, recent reports have shown that there is a close association between TJs and the stratum corneum (SC) and have suggested that the former is responsible for the formation of a complete SC barrier [4,5]. Specifically, patients suffering from claudin-1-null mutations show ichthyotic, xerotic, and flaky skin [5]. Moreover, claudin-1 is essential to filaggrin processing [4]; thus, claudin-1-knockout mice show decreased levels of filaggrin monomer, which is important for maintaining skin hydration and barrier integrity [2]. Similarly, elderly individuals show substantially reduced expression of filaggrin protein, but not mRNA, in the skin [6]. Expression profiles of epidermal TJ proteins associated with aging or UV irradiation have been controversial. While some investigators have reported that claudin-1 is increased after single UVB irradiation of 7.5 minimal erythema dose to hairless mice [7], others have shown that UVB irradiation does not change claudin-1 expression in cultured keratinocytes [8]. In contrast, claudin-1 expression is decreased following UV irradiation for 5 consecutive days in 24-week-old mice [9]. Furthermore, Rachow et al. showed that sun-exposed skin exhibits reduced expression of claudin-1, particularly in the lowermost epidermal layers [10]. However, they used clinical samples from participants of varying ages (sun-exposed skin, age 16–89 years, mean 67 years; sun-protected skin, age 20–78 years, mean 42 years) and locations (sun-exposed skin from face and forearm; sun-protected skin from abdomen) and did not show actual immunostaining images. We demonstrated changes in TJ proteins in homogenous age groups and locations and compared photoaged skin with intrinsically aged skin from the same individuals. Moreover, to avoid possible misleading effects

Abbreviations: TJ, tight junction; IF, immunofluorescence staining.